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Otolith Analysis of *Trachinotus ovatus* (L.) with Special Reference to Age

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Abstract. Otolith analysis of *Trachinotus ovatus* (L.) has been investigated to determine its crystal structure and some elemental composition with special reference to age. Results revealed that fish otoliths is composed of the aragonite form. Calcium was the most abundant element and showed different concentrations from one age group to another. Strontium was relatively easy to be incorporated into otolith. Chemical analysis of otolith for strontium-calcium concentrations ratio demonstrated changes in chemistry related to temperature. Ash percentage increased with increasing age. However, carbon and nitrogen percentage decreased with increasing age. It was concluded that there is strong relationships between otolith composition and age of fish.

Introduction

Fish otoliths are made of inorganic materials, generally calcium carbonate in the form of aragonite crystals (Carlstrom, 1963) which are deposited in a rhythmic fashion (Bagenal, 1974). The otoliths of fish contain a large amount of ecological data that may be accessible if the proper analytical techniques are employed (Radtke, 1989). In this regard, otoliths may serve as data storage units. By understanding the mechanisms of rhythmicity of carbonate deposition and the chemical patterns in otoliths, otoliths could be useful as metabolic, biological and ecological tools (Gauldie *et al.,* 1980; Pawson, 1990; Kalish, 1991a). However, these studies were performed for practical goals and relatively little information concerning otolith analysis with special reference to age is available. Therefore, the main objective of this study is to determine the relationship between age and otolith composition for *Trachinotus ovatus* (L).

Materials and Methods

Specimens of *Trachinotus ovatus* (L.) were collected from the commercial catch of purse-seine and beach seine landed at Alexandria from the Egyptian Mediterranean Sea.

Total length and gutted weight of each specimen were recorded. Sagittal otoliths were collected and stored dry in labelled envelopes for further study. Age was determined from the whole otolith against black background, using reflected light and viewed with a low power binocular microscope. Counts of zones were used to determine age as judged by the number of completed opaque zones. A part of each fish otoliths was finely ground and these powdered samples were submitted for X-ray diffraction analysis [Philips PW 1840 diffractometer] to determine the otolith crystal form under the following operating conditions: Scanning area from 25-60º θ, diffractometer speed º2 θ/ second, paper speed of the chart recorder 10 min/º2 θ, range of the recorder 1.04 counts/ second, time constant of signal to chart recorder 1 second and receiving slit width 0.3 mm. Calcium and strontium elements in the whole otolith were determined by Atomic Absorption Spectrometry [Spectr AA 10-plus]. Nitrogen content was determined by the Kjeldahl technique. Carbon content was measured by a modified Walkley-Black titration method (Gaudette and Flight, 1974). Ash residue was determined by placing the otoliths in a muffle furnace at 640ºC for 24 hr. T-test was used for statistical evaluation. Significance was accepted at $P < 0.05$.

Results and Discussion

In the X-ray diffraction pattern of otoliths samples, fifteen strong peaks appeared predominantly at angles of diffraction of 22.66º, 27.60º, 33.45º, 36.50º, 37.65º, 38.20º, 38.80º, 41.50º, 43.25º, 46.15º, 48.87º, 50.60º, 52.78º, 53.28º and 59.65º. The peaks 26.66º and 46.15º were far stronger than others. As shown in Fig. (1), these peaks are very narrow and sharp indicating a well crystallized form and belong to one mineral which is aragonite. The obtained results are in agreement with Carlstrom (1963); Degens *et al.* (1959); Gauldie and Nelson (1988) who demonstrated that the majority of fish otoliths are composed of fine prismatic aragonite crystals precipitated into a protein matrix.

Ideally, the composition of the calcium carbonate in otoliths would be thought to be unadulterated. However, there are a number of ions which could contaminate the aragonite, and three position for these ions have been suggested (Amiel *et al.*, 1973). Within the aragonitic crystals strontium can be a contaminant at very low levels and interchange with calcium in otoliths in the depositional processes.

Table (1) represents the relationship between age groups and calcium-strontium concentrations in the otoliths. It is evident that these elements showed a gradual increase in the mean concentrations with increasing age, *e.g.* the mean calcium content in the whole otolith of fish aged as 1-group was 876.4 µg/otolith. This mean increased in fish aged as 2-, 3-, 4- group to 66.0%, 91.7% and 95.5%, respectively. The mean strontium concentrations increased and showed the same trend. These results are in accordance with Casselman (1974) who found that calcium and total mineral content of hard tissue of pike *Esox lucius* (L.) increased with increasing age.

As shown in Table (1), the strontium/calcium ratios for different age groups were about 0.010497, 0.010515, 0.010476 and 0.010504 respectively. These low ratios suggests that otoliths are composed of almost pure aragonite crystals (Arai *et al.*, 1995).

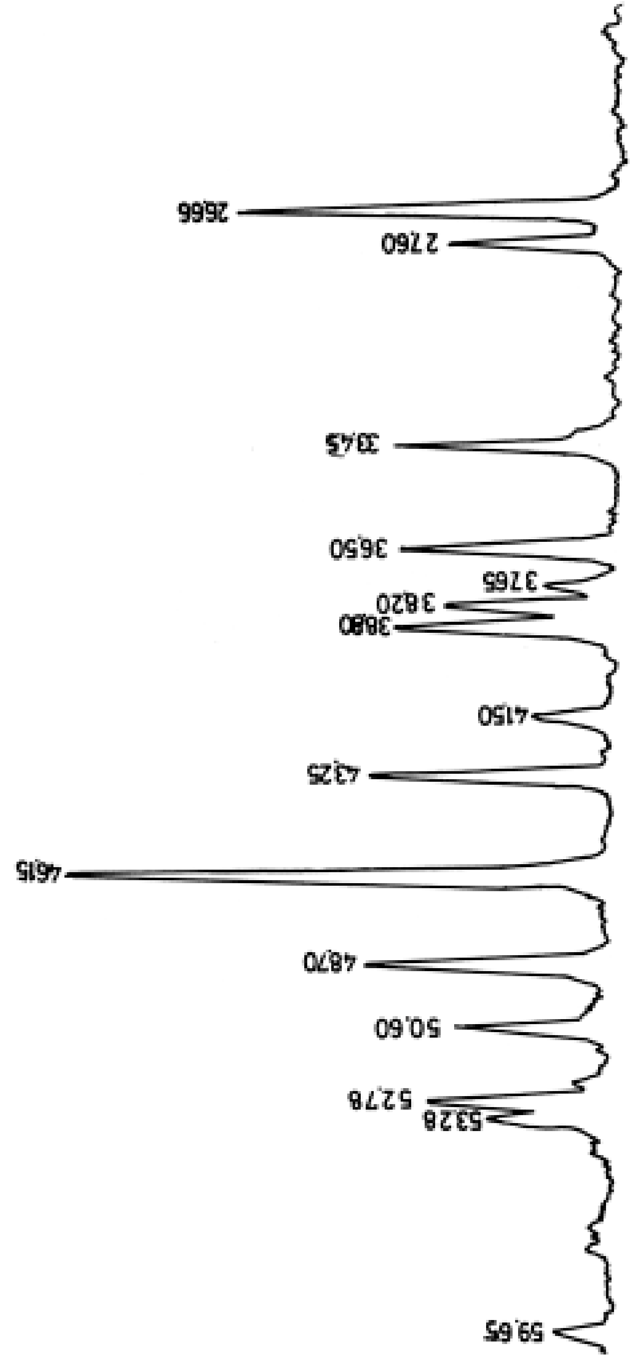

FIG. 1. X-ray diffraction pattern of *Trahinotus ovatus* otolith.

TABLE 1. The relationship between age groups and strontium-calcium concentration (µg/otolith) in the whole otolith of *Trachinotus ovatus* (L.).

Age group	Otolith weight	Ca^{2+} content	Sr^{2+} content	Sr/Ca $\times 10^{-3}$
I	2.4 ± 0.2	876.4 ± 22.9	9.2 ± 0.4	10.497
II	4.4 ± 0.2	1455.0 ± 43.3*	15.3 ± 0.5*	10.515
III	5.3 ± 0.3	1680 ± 43.7*	17.6 ± 0.6*	10.476
IV	6.6 ± 0.3	1713.6 ± 24.4	18.0 ± 1.0	10.504

Average of 12 fish ± standard deviation.

*Significant differences from next youngest age group.

As ambient seawater contains 400 mg/l calcium and 8 mg/l strontium (Nozaki, 1992), the Sr/Ca ratio is 0.02 in sea water by weight. It is well known that strontium can be substituted for calcium in the process of absorption of essential elements since strontium has a similar ionic radius (Ca: 0.99 Aº; Sr: 1.13 Aº) with the same 2+ valence and has similar properties in chemical reactions. Therefore, marine animals are thought to be able to discriminate between Sr and Ca. This is also supported by the result that observed Sr/Ca ratios in otoliths of *Trachinotus ovatus* are much smaller than that of seawater (0.010497, 0.010515, 0.010476 and 0.010504 for different age groups compared to seawater of 0.02). The obtained results are in accordance with Mugiya and Tanaka (1995) who suggested that cells concerning strontium transport discriminate strontium against calcium at both levels of strontium uptake from the ambient water and strontium transport from plasma to otoliths.

In the present study, we tested also the otolith Sr/Ca temperature relationship using fish aged as 3-group (assigned by otolith structure interpretation). As shown in Table (2), there is a negative correlation of mean otolith Sr/Ca ratio to ambient temperature (a = 14.68, b = – 0.17, 4 = – 0.99). This means that high Sr/Ca ratios should indicate low environmental temperatures and low Sr/Ca ratios should indicate high environmental temperatures. Consequently, temperature can be interpreted from chemical composition given the Sr/Ca concentrations ratios. The obtained results are in accordance with Smith *et al.* (1979) and Radtke (1989). However, it is premature to rule out the effect of growth since fish are poikilotherms and their growth is directly related to temperature. It seems feasible and is intuitive that temperature rate are linked (Sadovy and Severin, 1997).

The relationship between age groups and ash, nitrogen, carbon percentage are shown in Table (3). It is evident that ash percentage increased with increasing age, *e.g.* ash percentage for group 1+ was 53.9% while for groups 2+, 3+ and 4+ were 66.7, 73.3 and 78.1%, respectively. The obtained results support the inorganic content observation of the fish otolith (Table 1) which also increased with age. Dollerup (1964) showed that the increase in inorganic content of normal human bone with increasing ash was correlated with an increase in calcium content. This relationship occurs in different classes of animals (Dickerson, 1962), substantiating that it is a common phenomenon.

TABLE 2. The relationship between strontium calcium concentrations ratios (µg/otolith) and temperature in the whole otolith of *Trachinotus ovatus P(L.*) aged as group 3+.

Season	Temperature	Ca^{2+} content	Sr^{2+} content	$Sr/Ca \times 10^{-3}$
Winter	17.1 ± 0.4	1453.8 ± 41.9	17.0 ± 0.7	11.693
Spring	19.1 ± 0.8	1543.3 ± 49.2*	17.5 ± 0.8	11.339
Summer	27.2 ± 0.7	1839.3 ± 26.9*	18.2 ± 1.3	9.895
Autumn	24.0 ± 0.6	1680.2 ± 27.6*	17.8 ± 1.5	10.594

Average of 8 fish ± standard deviation.

*Significant differences from previous season.

TABLE 3. The relationship between age groups and percentage of ash, nitrogen and carbon in the whole otolith of *Trachinotus ovatus* (L.).

Age group	Ash (%)	Nitrogen (%)	Carbon (%)
I	53.9 ± 4.9	1.69 ± 0.30	0.06 ± 0.01
II	66.7 ± 3.4*	1.03 ± 0.12*	0.04 ± 0.02
III	73.3 ± 4.4*	0.76 ± 0.09*	0.03 ± 0.01
IV	78.1 ± 2.3*	0.64 ± 0.11*	0.02 ± 0.01

Average of 8 fish ± standard deviation.

*Significant differences from previous age group.

On the other hand, carbon content of the fish otolith decreased with increasing age (0.06, 0.04, 0.03 and 0.02%, respectively). However, measurement of carbon in fish otolith indicates that there is significant fractionation and carbon is deposited out of equilibrium (Kalish, 1991b). Nitrogen content decreased also with increasing age (1.69, 1.03, 0.76 and 0.64%, respectively). A decrease in the percentage of nitrogen and an increase in the percentage of inorganic constituents during growth and development have been reported for other animals (Widdowson and Dickerson, 1964). Several workers have considered a decrease in protein production important in annulus formation in the calcified tissue of fish, *e.g.* Keeton (1965) showed that marginal growth on scales may not be reduced while the rate of protein deposition is retarded or completely stopped, but that calcification of the edge continues.

Conclusions

1 – Otolith of *Trachinotus ovatus* (L.) is composed of calcium carbonate in the aragonite crystal form.

2 – There is a relationship between age of fish and otolith elemental composition.

3 – The relationship between quantity of organic and inorganic components of the fish otolith is inverse.

4 – Any condition which affects general body metabolism such as ambient temperature can be expected to be reflected in a change in the otolith composition.

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تحليـل عظمـة الأذن لسمكة الغلنفيش وعـلاقتـه بالعمر

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المستـخلص . تشـتـمل هـذه الدراسـة عـلى التـركـيب البـلوري وبعض المكونات العضـوية والغير عضوية لعظمـة الأذن في سمكة الغلنفـيش وقد أوضحت نتائج الدراسة ما يلي :

١- التركيب البلوري لعظمة الأذن في سمكة الغلنفيش هو الأراجونيت .

٢- هناك علاقة واضحة بين عمر السمكة ومكونات عظمة الأذن .

٣- العـلاقـة بين تركـيزات الكالسـيوم والأسـترونشـيوم في عظمـة الأذن لسمكة الغلنفيش بالنسبة للعمر كانت طردية .

٤- النسـبة بين عنصـري الأسترنشـيوم والكالسـيوم كـانت تتناسب عكسيًا مع زيادة درجات الحرارة .

٥- العـلاقـة بين نسـبة مكـونات النيتـروجين والكربون العضـوي كـانت عكسية بالنسبة لعمر الأسماك .